

What is claimed is:

1. A non-naturally occurring compound, comprising at least one  $\alpha$ 1,4-linked *N*-acetylglucosamine ( $\alpha$ 1,4-linked GlcNAc) residue operatively linked to a carrier molecule.
2. The compound of claim 1, wherein the carrier molecule comprises an *O*-glycan.
3. The compound of claim 2, wherein the *O*-glycan is a core2-branched *O*-glycan.
4. The compound of claim 1, wherein the carrier molecule comprises a polypeptide.
5. The compound of claim 4, wherein the polypeptide further comprises at least one *O*-glycosylation site.
6. The compound of claim 4, wherein the polypeptide comprises an *O*-glycan.
7. The compound of claim 5, wherein the *O*-glycan is a core2-branched *O*-glycan.
8. The compound of claim 1, wherein the carrier molecule comprises a mucin-like polypeptide.
9. The compound of claim 8, wherein the mucin-like polypeptide comprises CD43 (leukosialin), CD34, or Muc6.
10. The compound of claim 5, wherein the *O*-glycan is a core1 *O*-glycan.
11. The compound of claim 1, comprising a plurality of  $\alpha$ 1,4-linked GlcNAc residues.

12. The compound of claim 1, comprising a synthetic oligosaccharide.
13. A method of producing a glycoprotein comprising at least one  $\alpha$ 1,4-linked *N*-acetylglucosamine ( $\alpha$ 1,4-linked GlcNAc) residue, comprising contacting, under conditions suitable for glycosylation of a polypeptide,
  - an  $\alpha$ 1,4-*N*-acetylglucosaminyl transferase ( $\alpha$ 4GnT),
  - a core2  $\beta$ 1,6-*N*-acetylglucosaminyl transferase-I (C2GnT-I) or a core1 extension  $\beta$ 1,3-*N*-acetylglucosaminyl transferase (C1- $\beta$ 3GnT), and
  - a carrier polypeptide, which comprises at least one *O*-glycosylation site,whereby the carrier polypeptide is glycosylated by the C2GnT-I or by the C1- $\beta$ 3GnT, and the  $\alpha$ 4GnT, thereby producing a glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue.
14. The method of claim 13, wherein the carrier polypeptide is a soluble polypeptide.
15. The method of claim 13, wherein the carrier polypeptide is CD43 (leukosialin), CD34, or Muc6.
16. The method of claim 13, wherein the carrier polypeptide is a mucin-type glycoprotein secreted in milk of a mammal.
17. The method of claim 16, which comprising contacting milk containing the mucin-type glycoprotein with the  $\alpha$ 4GnT and, optionally, the C2GnT-I or the C1- $\beta$ 3GnT.
18. The method of claim 13, wherein the glycoprotein comprises a plurality of  $\alpha$ 1,4-linked GlcNAc residues.
19. The method of claim 13, further comprising isolating the glycoprotein.

20. A glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue, said glycoprotein produced by the method of claim 13.

21. Milk produced by the method of claim 17, wherein the milk contains a glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue.

22. A method of producing a recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked *N*-acetylglucosamine ( $\alpha$ 1,4-linked GlcNAc) residue, comprising expressing in a eukaryotic cell,

a first polynucleotide encoding a  $\alpha$ 1,4-*N*-acetylglucosaminyl transferase ( $\alpha$ 4GnT),

a second polynucleotide encoding a core2  $\beta$ 1,6-*N*-acetylglucosaminyltransferase-I (C2GnT-I) or encoding a core1 extension  $\beta$ 1,3-*N*-acetylglucosaminyl transferase (C1- $\beta$ 3GnT), and

a third polynucleotide encoding a carrier polypeptide comprising at least one *O*-glycosylation site,

wherein at least one of the first polynucleotide, second polynucleotide, and third polynucleotide is an exogenous polynucleotide introduced into the eukaryotic cell,

whereby the carrier polypeptide is glycosylated by the C2GnT-I or by the C1- $\beta$ 3GnT, and the  $\alpha$ 4GnT, thereby producing a recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue.

23. The method of claim 22, wherein the carrier polypeptide is a soluble polypeptide.

24. The method of claim 22, wherein the carrier polypeptide is CD43 (leukosialin), CD34, or Muc6.

25. The method of claim 22, wherein the recombinant glycoprotein comprises a plurality of  $\alpha$ 1,4-linked GlcNAc residues.

26. The method of claim 22, wherein the eukaryotic cell is a mammalian cell.
27. The method of claim 22, further comprising isolating the recombinant glycoprotein.
28. A recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue, said recombinant glycoprotein produced by the method of claim 22.
29. An isolated recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue, said isolated recombinant glycoprotein produced by the method of claim 27.
30. A non-human transgenic mammal containing, stably integrated in its genome,  
at least first exogenous polynucleotide encoding an  
 $\alpha$ 1,4-*N*-acetylglucosaminyl transferase ( $\alpha$ 4GnT);  
a second polynucleotide encoding a core2  
 $\beta$ 1,6-*N*-acetylglucosaminyltransferase-I (C2GnT-I) or encoding a core1 extension  
 $\beta$ 1,3-*N*-acetylglucosaminyl transferase (C1- $\beta$ 3GnT); and  
a third polynucleotide encoding a carrier polypeptide comprising at least  
one *O*-glycosylation site,  
wherein at least one of the first polynucleotide, second polynucleotide, and third  
polynucleotide is an exogenous polynucleotide;  
wherein the first polynucleotide, second polynucleotide, and third polynucleotide  
are operatively linked to a 5' regulatory sequence of a mammary gland-specific gene  
including a promoter; and  
wherein the third polynucleotide is operatively linked to a nucleotide sequence  
encoding a signal sequence effective in directing secretion of the carrier polypeptide into  
milk;  
whereby, upon expression of the C2GnT-I or the C1- $\beta$ 3GnT, the  $\alpha$ 4GnT, and the  
carrier polypeptide, the carrier polypeptide is glycosylated by the C2GnT-I or by the

C1- $\beta$ 3GnT, and the  $\alpha$ 4GnT, thereby producing a recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue.

31. The transgenic non-human mammal of claim 30, wherein at least one of second polynucleotide and the third polynucleotide comprises an exogenous polynucleotide.

32. The transgenic non-human mammal of claim 30, wherein each of the first polynucleotide, second polynucleotide, and third polynucleotide is an exogenous polynucleotide.

33. The transgenic mammal of claim 30, wherein the promoter comprises a whey acidic protein (WAP) promoter, an  $\alpha$ -casein promoter, a  $\beta$ -casein promoter, a  $\kappa$ -casein promoter, an  $\alpha$ -lactalbumin promoter, or a  $\beta$ -lactoglobulin promoter.

34. The transgenic mammal of claim 30, wherein the first polynucleotide, second polynucleotide, and third polynucleotide are further operatively linked to 3' regulatory sequences from a mammary gland-specific gene or 3' regulatory sequences active in a mammary gland.

35. The transgenic mammal of claim 34, wherein each of the first polynucleotide, second polynucleotide, and third polynucleotide is operatively linked to said 5' regulatory sequence and said 3' regulatory sequence.

36. The transgenic mammal of claim 34, wherein the first polynucleotide, second polynucleotide, and third polynucleotide comprise a single, operatively linked nucleic acid molecule, which is operatively linked to said 5' regulatory sequence and said 3' regulatory sequence.

37. The transgenic mammal of claim 30, wherein the mammal is a mouse, a rat, a rabbit, a horse, a pig, a sheep, a goat, or a cow.

38. The transgenic mammal of claim 30, which is a female non-human transgenic mammal, wherein the recombinant glycoprotein is secreted in an antimicrobially active form into milk produced by said female non-human transgenic mammal.

39. Milk produced by the female non-human transgenic mammal of claim 38.

40. A method for producing an antimicrobial recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue in milk of a female non-human transgenic mammal, comprising:

a) introducing into a non-human mammalian embryo at least a first exogenous polynucleotide encoding an  $\alpha$ 1,4-*N*-acetylglucosaminyl transferase ( $\alpha$ 4GnT);

wherein the first exogenous polynucleotide is operatively linked to a

5' regulatory sequence of a mammary gland-specific gene including a promoter;

whereby, upon expression of the  $\alpha$ 4GnT a carrier polypeptide in mammary epithelial cells is glycosylated by the  $\alpha$ 4GnT, thereby producing an antimicrobial recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue;

b) transferring the embryo of a) into a recipient female mammal such that progeny are produced;

c) inducing milk production in a female progeny of b) containing the first exogenous polynucleotide operatively linked to the 5' regulatory sequence stably integrated its genome,

thereby producing an antimicrobial recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue in the milk of the female non-human transgenic mammal.

41. The method of claim 40, further comprising introducing into the non-human mammalian embryo

- a) a second exogenous polynucleotide encoding an exogenous core2  $\beta$ 1,6-*N*-acetylglucosaminyltransferase-I (C2GnT-I) or encoding an exogenous core1 extension  $\beta$ 1,3-*N*-acetylglucosaminyl transferase (C1- $\beta$ 3GnT); or
- b) a third exogenous polynucleotide encoding an exogenous carrier polypeptide comprising at least one *O*-glycosylation site; or
- c) the second exogenous polynucleotide and the third endogenous polynucleotide,

wherein the second exogenous polynucleotide, when present, the third exogenous polynucleotide, when present, or second exogenous polynucleotide and third exogenous polynucleotide, when present, is operatively linked to a 5' regulatory sequence of a mammary gland-specific gene including a promoter; and

wherein the third exogenous polynucleotide, when present, is operatively linked to a nucleotide sequence encoding a signal sequence effective in directing secretion of the exogenous carrier polypeptide into milk,

whereby, upon expression of the C2GnT-I or the C1- $\beta$ 3GnT, when present, the  $\alpha$ 4GnT, and the exogenous carrier polypeptide, when present, the carrier polypeptide is glycosylated by the C2GnT-I or by the C1- $\beta$ 3GnT, when present, and the  $\alpha$ 4GnT, thereby producing an antimicrobial recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue.

42. The method of claim 40, further comprising milking the female progeny of c), thereby obtaining milk containing the recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue.

43. The method of claim 42, further comprising isolating the recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue from the milk, thereby obtaining the recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue.

44. Milk obtained by the method of claim 42.
45. An isolated recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue obtained by the method of claim 43.
46. A method of reducing or inhibiting cell wall formation of a bacterium that expresses cholesteryl- $\alpha$ -D-glucopyranoside (CGL), comprising contacting the bacterium with a compound comprising at least one  $\alpha$ 1,4-linked GlcNAc residue, whereby CGL synthesis is reduced or inhibited, thereby reducing or inhibiting cell wall formation of the bacterium.
47. The method of claim 46, wherein the compound reduces or inhibits UDP-Glc:sterol glucosyltransferase activity in the gram negative bacterium.
48. The method of claim 46, wherein the bacterium is a *Helicobacter* species.
49. The method of claim 48, wherein the *Helicobacter* species is *Helicobacter pylori*.
50. The method of claim 46, wherein the compound comprises isolated gastric gland mucous cell-type mucin.
51. A method of reducing or inhibiting growth of bacteria expressing cholesteryl- $\alpha$ -D-glucopyranoside (CGL), comprising contacting the bacteria with a compound comprising at least one  $\alpha$ 1,4-linked *N*-acetylglucosamine ( $\alpha$ 1,4-linked GlcNAc), whereby CGL synthesis is reduced or inhibited, thereby reducing or inhibiting growth of the bacterium.
52. The method of claim 51, wherein the compound reduces or inhibits UDP-Glc:sterol glucosyltransferase activity in the bacteria.



53. The method of claim 51, wherein the bacteria is a *Helicobacter* species.
54. The method of claim 53, wherein the *Helicobacter* species is *Helicobacter pylori*.
55. The method of claim 51, wherein the compound comprises isolated gastric gland mucous cell-type mucin.
56. The method of claim 51, wherein the compound comprises an *O*-glycan.
57. The method of claim 51, wherein the compound comprises a synthetic oligosaccharide.
58. A method of ameliorating signs or symptoms of a gastric ulcer due to a *Helicobacter* species infection in a subject, comprising administering to the subject a compound comprising at least one  $\alpha$ 1,4-linked *N*-acetylglucosamine ( $\alpha$ 1,4-linked GlcNAc) residue, whereby the compound, upon contacting the *Helicobacter*, reduces or inhibits growth of the *Helicobacter* species, thereby ameliorating signs or symptoms of the gastric ulcer.
59. The method of claim 58, wherein the *Helicobacter* species is *Helicobacter pylori*.
60. The method of claim 58, wherein the subject is a mammal.
61. The method of claim 58, wherein the mammal is a cow, a horse, a pig, a goat, a dog, a cat, or a ferret.
62. The method of claim 58, wherein the subject is a human.

63. The method of claim 58, wherein the compound comprises isolated gastric gland mucous cell-type mucin.

64. The method of claim 58, wherein the compound comprises an *O*-glycan comprising a terminal  $\alpha$ 1,4-linked GlcNAc.

65. The method of claim 58, wherein the compound comprises soluble CD43 (leukosialin) comprising a terminal  $\alpha$ 1,4-linked GlcNAc.

66. The method of claim 58, wherein the compound comprises a synthetic oligosaccharide.

67. The method of claim 58, wherein the compound is administered orally.

68. The method of claim 67, which comprises drinking the milk of claim G4.

69. A method of preventing gastric ulcers due to infection by a *Helicobacter* species in a subject susceptible to the gastric ulcers, comprising administering to the subject a compound comprising at least one  $\alpha$ 1,4-linked *N*-acetylglucosamine ( $\alpha$ 1,4-linked GlcNAc) residue, whereby the compound prevents *Helicobacter* growth, thereby preventing gastric ulcers due to infection by the *Helicobacter*.

70. The method of claim 69, wherein administering the compound comprises the subject drinking the milk of claim 21.

71. A method of ameliorating gastric ulcers or gastric cancer due to infection by a *Helicobacter* species in a subject having gastric ulcers or gastric cancer, comprising administering to the subject a compound comprising at least one  $\alpha$ 1,4-linked *N*-acetylglucosamine ( $\alpha$ 1,4-linked GlcNAc) residue, whereby the compound prevents *Helicobacter* growth, thereby ameliorating gastric ulcers or gastric cancer due to infection by the *Helicobacter*.

72. The method of claim 71, wherein administering the compound comprises the subject drinking the milk of claim 21.

73. A transgenic plant containing, stably integrated in its genome,  
at least first exogenous polynucleotide encoding an  
 $\alpha$ 1,4-*N*-acetylglucosaminyl transferase ( $\alpha$ 4GnT);  
a second polynucleotide encoding a core2  
 $\beta$ 1,6-*N*-acetylglucosaminyltransferase-I (C2GnT-I) or encoding a core1 extension  
 $\beta$ 1,3-*N*-acetylglucosaminyl transferase (C1- $\beta$ 3GnT); and  
a third polynucleotide encoding a carrier polypeptide comprising at least  
one *O*-glycosylation site,  
wherein at least one of the first polynucleotide, second polynucleotide, and third  
polynucleotide is an exogenous polynucleotide; and  
wherein the first polynucleotide, second polynucleotide, and third polynucleotide  
are operatively linked to a plant gene 5' regulatory sequence, including a promoter;  
whereby, upon expression of the C2GnT-I or the C1- $\beta$ 3GnT, the  $\alpha$ 4GnT, and the  
carrier polypeptide, the carrier polypeptide is glycosylated by the C2GnT-I or by the  
C1- $\beta$ 3GnT, and the  $\alpha$ 4GnT, thereby producing a recombinant glycoprotein comprising at  
least one  $\alpha$ 1,4-linked GlcNAc residue.

74. The transgenic plant of claim 73, wherein at least one of second  
polynucleotide and the third polynucleotide comprises an exogenous polynucleotide.

75. The transgenic plant of claim 73, wherein each of the first polynucleotide, second polynucleotide, and third polynucleotide is an exogenous polynucleotide.

76. The transgenic plant of claim 73, wherein the promoter comprises a constitutive promoter, an inducible promoter, or a tissue specific promoter.

77. The transgenic plant of claim 73, wherein the promoter comprises a 35S cauliflower mosaic virus promoter, an ubiquitin promoter, a tetracycline responsive promoter, an *rbcS* gene light inducible promoter, a seed specific oleosin gene promoter, or a rice actin promoter.

78. The transgenic plant of claim 73, wherein the plant is soybean or rice.

79. Seeds produced by the transgenic plant of claim 73.

80. Grain produced from the transgenic plant of claim 73.

81. A milk produced from the transgenic plant of claim 73.

82. A method for producing an antimicrobial recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue in a transgenic plant, comprising:

a) introducing into a plant cell at least a first exogenous polynucleotide encoding an  $\alpha$ 1,4-*N*-acetylglucosaminyl transferase ( $\alpha$ 4GnT);

wherein the first exogenous polynucleotide is operatively linked to a 5' regulatory sequence of a plant gene, including a promoter;

whereby, upon expression of the  $\alpha$ 4GnT, a carrier polypeptide in cells of the plant is glycosylated by the  $\alpha$ 4GnT, thereby producing an antimicrobial recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue; and

b) growing a plant from the plant cell of a), thereby obtaining a transgenic plant, whereby an antimicrobial recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue is produced in the transgenic plant.

83. The method of claim 82, further comprising introducing into the plant cell

a) a second exogenous polynucleotide encoding an exogenous core2  $\beta$ 1,6-*N*-acetylglucosaminyltransferase-I (C2GnT-I) or encoding an exogenous core1 extension  $\beta$ 1,3-*N*-acetylglucosaminyl transferase (C1- $\beta$ 3GnT); or

b) a third exogenous polynucleotide encoding an exogenous carrier polypeptide comprising at least one *O*-glycosylation site; or

c) the second exogenous polynucleotide and the third endogenous polynucleotide,

wherein the second exogenous polynucleotide, when present, the third exogenous polynucleotide, when present, or second exogenous polynucleotide and third exogenous polynucleotide, when present, is operatively linked to a 5' regulatory sequence of a plant gene, including a promoter;

whereby, upon expression of the C2GnT-I or the C1- $\beta$ 3GnT, when present, the  $\alpha$ 4GnT, and the exogenous carrier polypeptide, when present, the carrier polypeptide is glycosylated by the C2GnT-I or by the C1- $\beta$ 3GnT, when present, and the  $\alpha$ 4GnT, thereby producing an antimicrobial recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue in the transgenic plant.

84. The method of claim 82, further comprising isolating a transgenic plant product comprising at least one of seeds, leaves, roots, or flowers from the transgenic plant.

85. The method of claim 84, further comprising isolating the recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue from the transgenic plant product, thereby obtaining the recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue.

86. The method of claim 84, further comprising processing the transgenic plant product to obtain a milk product comprising the recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue.

87. A recombinant glycoprotein comprising at least one  $\alpha$ 1,4-linked GlcNAc residue produced by the method of claim 85.

88. Milk obtained by the method of claim 86.